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ALVEOLAR LIQUID LINING: LANGMUIR METHOD USED TO MEASURE SURFACE TENSION IN BOVINE AND CANINE LUNG EXTRACTS

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SUMMARY

1. The Langmuir trough has been used to study monolayers of surfactant from beef lung extracts, dog lavage and the principal component (dipalmitoyl lecithin; DPL) in which surface tension has been simultaneously monitored on each film by the Wilhelmy method and the original Langmuir method whose readings are independent of contact angle.

2. Readings on the Wilhelmy balance at 80 % film compression reached the near-zero values recorded in previous studies, averaging 2·0 dyn cm^{-1}, but Langmuir readings *on the same films* averaged 13·5 dyn cm^{-1} for DPL with no reading below 7·0 dyn cm^{-1}.

3. Similar differences were found for bovine extracts and dog lung lavage and have been attributed to a serious contact-angle artifact in the Wilhelmy method widely used in studying pulmonary surfactant.

4. Higher minimum values of surface tension determined by the Langmuir method are shown to be incompatible with normal physiological function in the smaller mammals on the basis of the traditional bubble model of the alveolus.

5. When Langmuir or Wilhelmy values are determined under more physiological conditions of area change, pH, temperature, humidity etc., values for surface tension are even higher and less compatible with the concept of a continuous liquid lining for the alveolar diameter of any mammal.

INTRODUCTION

There has been no doubt since the first studies of von Neergaard (1929) who inflated the lung with liquid that the alveolar surface plays a major role in pulmonary mechanics and also in those aspects of homoeostasis involving interfacial forces acting upon the alveolus. In the interpretation of those and many later studies, it has been generally assumed that there is a continuous liquid lining to the lung which provides an air–aqueous interface on which surfactant can locate and exert its influence simply by changing surface contractile forces. The further assumption that this aqueous hypophase is continuous has proven most convenient since it has then enabled surface tension to be directly related by the Laplace equation to the pressure with which a surface would tend to collapse (ΔP) as though it were the inside of a bubble:

$$\Delta P = 2\gamma/r, \tag{1}$$

where γ is the surface tension and r is the radius of curvature. The benefits of surfactant are thus conventionally explained on the basis that it reduces this collapsing pressure, while liquid filling eliminates ΔP in eqn. (1) altogether, leaving tissue forces only (von Neergaard, 1929; Radford, 1957). However, liquid filling could also reduce the inflation pressure if the alveolar surface were dry or only partially wetted (Hills, 1982*a*, *b*).

In a careful analysis of many of the more detailed aspects of this bubble model of the alveolus, Tierney (1974) has pointed out that the curvature at the septal corners can exceed that which would apply if the alveolus were assumed to be spherical, when the lowest value of r in eqn. (1) would require much lower values of γ unless the collapsing pressure (ΔP) rises to unacceptable values. Even if the best conditions are assumed, i.e. the alveolus were spherical, it is difficult to envisage a collapsing pressure of less than 10 cmH_2O for small mammals such as the bat ($r = 14{\cdot}5$ μm) or shrew ($r = 16$ μm) unless γ is less than 7 dyn cm^{-1} (Hills, 1982*b*). Hence, in justifying the bubble model, great emphasis has been placed upon the experimental finding that such low surface tensions have indeed been recorded on the Langmuir trough using lung washings, extracts or the active components of lung surfactant (Clements & Tierney, 1965). Prior to these studies, Pattle (1958) had predicted these 'near-zero' values of surface tension based upon the remarkable stability of bubbles expressed from lung tissue.

However, it has been pointed out by Barrow & Hills (1979*b*) that the low values of surface tension needed to satisfy the bubble model have only been recorded on the Langmuir trough under conditions which would seem far from physiological. Most studies showing those low values have been conducted at room temperature and for compressions of the surface film which would seem grossly in excess of alveolar area changes likely to occur during ventilation. To be specific, surface tensions have been reported for compression to 20 % of initial area of 6–7 dyn cm^{-1} for purified dog lavage (Steim, Redding, Hauck & Steim, 1969), 1–5 dyn cm^{-1} for beef lung extract (Klaus, Clements & Havel, 1961), 10–15 dyn cm^{-1} (Frosonolo, Charms, Pawlowski & Slivka, 1970) or '15 dyn cm^{-1} or less' (Scarpelli, Chang & Colacicco, 1970) for dog extract, 6–12 dyn cm^{-1} for fetal rabbit extract (Gluck, Motoyama, Smits & Kulovich, 1967); while, at 15 % of initial area, lowest reported values are 4 dyn cm^{-1} for dog lavage (King & Clements, 1972*b*) or 'less than 10 dyn cm^{-1}' for rabbit lavage (Dickie, Massaro, Marshall & Massaro, 1972) but 'less than 10 dyn cm^{-1}' for dog extract reduced to 1·4 % of initial area (King & Clements, 1972*a*). Many other studies reviewed by Bangham, Morley & Phillips (1979) have shown 'near-zero' values but these were also recorded using the Wilhelmy balance or with a similar type of flag dipping into the pool of a Langmuir trough and, therefore, subject to underestimation of the true surface tension if there were a contact angle and this were ignored (Gaines, 1966; Hills & Ng, 1974). The standard argument against this objection has been that a perfectly clean platinum flag is perfectly wetted by water and will therefore give a contact angle of zero. This is true as far as it goes but the surfactant can be regarded as the impurity and, under conditions similar to those prevailing in the studies quoted above, has been claimed to produce large contact angles (Hills & Ng, 1974; Barrow & Hills, 1979*a*). In other studies where cognizance has been taken of this source of potential error, the contact angle has been assumed to be zero (Scarpelli, 1968) or, at a fixed pool area, found not to change with the variable under investigation (Mendenhall & Stokinger, 1962).

Since our basic concepts of the alveolus depend very heavily upon the true values of surface tension in the lung, it might seem appropriate to invoke measurement methods which avoid any possibility of contact-angle artifact. Earlier approaches have used the inflation pressure of an oscillating bubble (Enhorning, 1977), *in situ* measurements using fluorocarbon droplets (Schürch, Goerke & Clements, 1981) or the ring detachment principle of du Noüy (Barrow & Hills, 1979*a*). However, none of these are easily adapted to the Langmuir trough on which surface conditions can be well defined and maintained; while other objections to these methods are discussed later.

In this study it is proposed to return to the original method of Langmuir (1917) who measured surface tension by recording the horizontal force exerted by the film upon a floating boom. This classical method has not been used in surface physics for some decades due, largely, to the engineering problems, but these have now been overcome in a superb yet very expensive commercial instrument recently released. This apparatus not only avoids errors due to contact angle but retains what, to most physiologists, is the great advantage of the Wilhelmy balance by permitting continuous recording from one side of the pool of a Langmuir trough as another side is cycled back and forth to simulate alveolar area changes with ventilation.

This instrument has therefore been used in this study to determine (a) the surface area:surface tension relationship under simultaneously simulated physiological conditions and (b) whether, under less physiological conditions, surface tension really reaches the 'near-zero' values previously claimed on the basis of other methods.

METHODS

Apparatus

This study is made possible by the advent of the new surface balance manufactured by Lauda Messgeraete Werk, Lauda-Tauber, F.R.G. (model 1974). Superb engineering and the use of Teflon now overcome many of the practical problems associated with the Langmuir (1917) method when it was introduced at the beginning of the century. It is based upon a boom floating in a Langmuir trough and thus dividing the surface in two pools. A very sophisticated force transducer system then measures the differential horizontal pull on the boom proportional to the difference in surface tension between the first pool (A) behind the boom and pool 'B' in front of it. The analysis and experiments of Harkins & Anderson (1937) prove that this principle gives surface tension readings totally independent of any contact angle between the surface and the material of the boom; Teflon in this case. The output from the horizontal force transducer on the Lauda boom is fed into their film balance analyser (model A) and automatically plotted on an X–Y recorder (Brinkman model 733/42/42/51).

The pool in front of the barrier is divided into two by a moveable barrier to give a third pool (C) behind the barrier and pool 'B' which is the one on which the surfactant is 'touched off' and surface tension studied with change of area. In this study, a platinum flag was dipped into this pool (B) suspended from a force transducer (Statham model UC2) connected to a recorder (Gould model 2400S) to record the vertical pull according to the Wilhelmy (1863) principle. The force (F) recorded by the vertical transducer is dependent upon any contact angle (θ) and related to the true surface tension (γ) by the expression:

$$F = \gamma L \,.\, \cos(\theta), \tag{2}$$

where L is the wetted perimeter of the flag.

The comparison between 'Langmuir' and 'Wilhelmy' readings thus offers a very sensitive means of detecting any errors previously unappreciated in ignoring the contact angle, i.e. the 'cos θ' term in eqn. (2). This arrangement has the advantage over previous attempts by Barrow & Hills (1979*a*) to make the same comparison in that both techniques are recording from the same film simultaneously over the entire area cycle without any compensatory barrier movements being required.

Since some surfactant films can be fairly rigid, great care has been taken to prevent leaks between the pools or up the sides of the trough. At the exceptionally low surface tensions which might be obtained upon 75–80 % film compression, leaks are difficult to avoid but represent no ultimate error provided they are detected. This has been achieved with three Wilhelmy flags, one dipping into each of the three pools (A, B and C) into which the Langmuir trough is divided by the boom and the moveable barrier; see Pl. 1. Pool 'A' (behind the boom) is 172 cm^2 while, before compression, 'B' is 585 cm^2 and pool 'C' is 229·5 cm^2. The barrier was set at the fastest speed of 6·5 cm min^{-1} or 16·7 % of initial pool area per minute, corresponding to 3 min $cycle^{-1}$ for 25 % compression.

The platinum plates used were the standard flags (2·5 cm in length) taken from the Kimray–Greenfield version of the Wilhelmy balance popular in pulmonary studies.

Materials

The surfactants used for this study were extracts of minced bovine lung, dog lung lavage and a commercial extract of dipalmitoyl lecithin (DPL) long accepted as the most surface-active component of lung surfactant (Klaus *et al.* 1961; Frosonolo *et al.* 1970), at least with respect to the ability of such substances to reduce surface tension. Specifically, the DPL used was L-α-dipalmitoyl phosphatidylcholine (lot 31F-8350) supplied by the Sigma Chemical Company and kept refrigerated at 0 °C. The 99 % purity specified by the manufacturer was checked by thin-layer chromatography followed by spectrophotometric analysis of spots for phosphorus according to the method of Rouser, Siakotos & Fleischer (1966). Since this material was found to reduce the 'Wilhelmy' reading of apparent surface tension to the very low values under investigation, it was felt that nothing could be gained by striving for even better purity. The surfactant was dissolved in chloroform to a concentration of 55 $\mu g\ ml^{-1}$ such that 4 ml applied to the surface of pool 'B' bounded by the floating boom and the barrier gave a surface concentration of 0·38 $\mu g\ cm^{-2}$ (50 Å^2 per molecule) which is close to the upper limit estimated for physiological concentrations of total pulmonary surfactant (Barrow & Hills, 1979*b*). Compression to 80 % of initial area produces a condensed monomolecular layer assuming a molecular cross-sectional area of 40 Å^2 (Harkins & Anderson, 1937).

Procedure

The bovine lungs were placed in a blender within 2 h of death of the steer, macerated and the phospholipid extracted with chloroform according to the method of Folch, Lees & Sloane-Stanley (1957). The dry chloroform extract was redissolved in chloroform to the same concentration as the DPL, viz. 55 $\mu g\ ml^{-1}$.

Dog lungs were lavaged by standard procedures (Klaus *et al.* 1961), evacuating them and then re-inflating with saline to ensure that the liquid reached the alveoli. They were then continuously inflated and deflated gently with the same saline (1 l) to avoid blood contamination. Phospholipid was then extracted from this lavage fluid by chloroform extraction, again following the method of Folch *et al.* (1957). The dry chloroform extract was redissolved to a concentration of 55 $\mu g\ l^{-1}$.

All surfaces of the Langmuir trough and balance were kept scrupulously clean by rinsing in chloroform and acetone before drying. The three platinum flags were also flamed before each run and calibrated with weights. 735 ml saline buffered to a pH of 7·4 was then poured into the trough, the boom floated on the pool, the three Wilhelmy flags immersed and the barrier cycled to 15 % of initial area and back three times with no surfactant. If the reading on the boom or any of the three Wilhelmy transducers indicated a surface change of 1 dyn cm^{-1} or more, then the trough was drained and the whole cleaning procedure repeated until there was no detectable surface contamination. 4 ml of the chloroform solution of either lung extract of DPL were 'touched off' at the surface of pool 'B' and 15 min allowed for the chloroform to evaporate and deposit the surface film.

The barrier was then set in motion until the desired compression of the film was reached, when it was returned to 100 % of pool area at the same linear rate. Continuous recordings were taken of surface forces acting upon the floating boom and the three Wilhelmy flags. The process was repeated until the recordings were reproduced by subsequent cycles; a practical point to observe when considering the application of the results to ventilation with its repetitive area changes (Clements, Hustead, Johnson & Gribetz, 1961). These cycles were essentially stable by the third loop recorded, as found in previous studies (Brown, Johnson & Clements, 1959).

In the first series of experiments both DPL and bovine extract were compressed to 20 % of initial area at room temperature (21 ± 1 °C). Both third and first loops were recorded since, upon

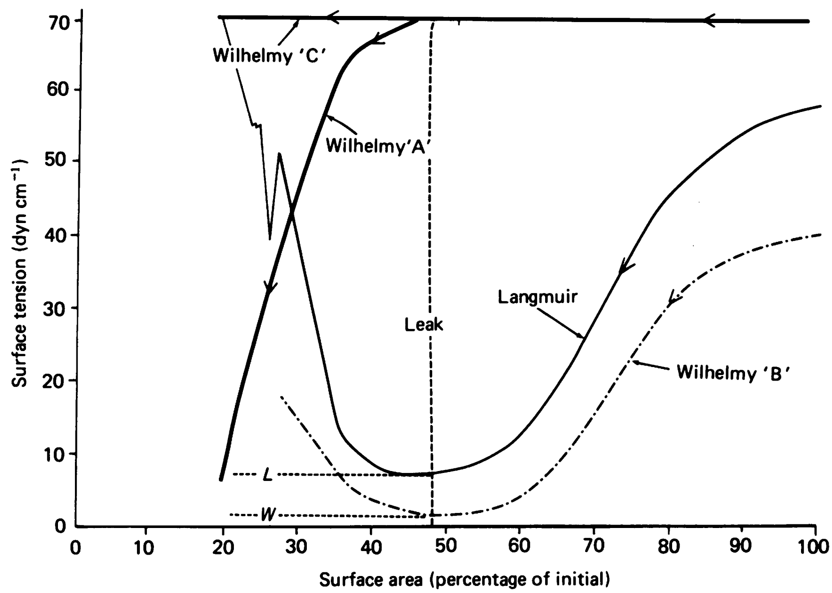

Fig. 1. Demonstrating how the four simultaneous monitors of the surface tension of a DPL film at 21 °C enable a leak to be detected immediately it occurs. In this instance surfactant leaked across the floating boom which lowered the differential force on the boom separating pools 'A' and 'B' and, hence, the Langmuir reading. This was confirmed by both the rise in the reading of the Wilhelmy flag in pool 'B' as it lost surfactant and the fall in Wilhelmy 'A' as pool 'A' received surfactant; while Wilhelmy 'C' remained constant because the moving barrier did not leak. *L* and *W* represent Langmuir and Wilhelmy readings respectively recorded simultaneously in subsequent leak-free runs just before film collapse (2 % larger area). Those results are given in Table 1.

compression, the surface tension tends to be lowered sooner on the first cycle. Any run was stopped as soon as a leak was detected. After runs showing no detectable leaks, pools 'A' and 'C' were compressed to 15 % of their original area to ensure that no surfactant had leaked around the floating boom or moveable barrier and failed to be detected immediately due to any film cohesion of the escaping surfactant.

Physiological conditions

In the second series, a number of parameters were changed to try to offer the best simulation of physiological conditions. These included heating the trough and surroundings to 37 ± 0·5 °C, humidifying the air above the trough at that temperature, maintaining a lung pH of 7·4 and selecting a compression limit of 100–75 % of initial area; the maximum likely in normal respiration (Pattle, 1977).

Third loops were recorded for films of DPL and an extract of dog lavage.

TABLE 1. Minimum surface tensions for DPL films at 21 °C

Run	Area* (% of initial)	Surface tension ($dyn\ cm^{-1}$) Wilhelmy (W)	Langmuir (L)	Contact angle (θ) $\cos^{-1}(W/L)$
No. 1	48	3·0	9	71 deg
No. 2	58	2·0	23	85 deg
No. 3	32	2·9	8	69 deg
No. 4	56	3·1	20	81 deg
No. 5	48	1·1	12	85 deg
No. 6	60	2·8	7	66 deg
No. 7	54	0·4	8	86 deg
No. 8	56	0·3	7	88 deg
Means ± S.E. of mean		2·0 ± 0·4	13·5 ± 2·3	78·9 ± 3·1 deg

* Area 2 % lower than film collapse and the area to which both Wilhelmy and Langmuir readings refer.

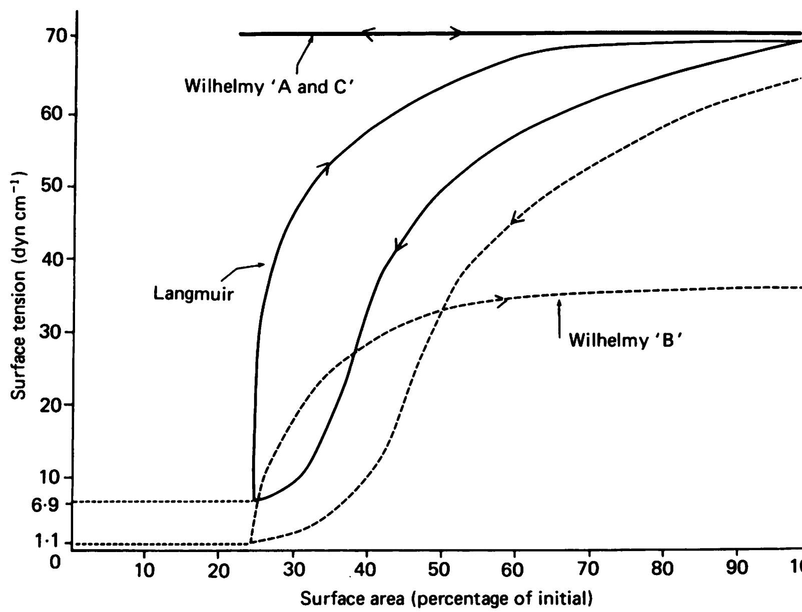

Fig. 2. The first loop for a DPL film for the conditions (35 °C) which enabled the lowest Langmuir reading (6·9 dyn cm^{-1}) to be recorded consistently without film collapse. Note the absence of leaks by the constancy of the Wilhelmy flags in pools 'A' and 'C' (70 dyn cm^{-1}), which also failed to change upon subsequent compression of those pools by 85 %.

RESULTS

In no case could the meniscus of the pool be seen to rise to the top of the walls of the trough and barrier nor was the moving barrier found to leak when the pool 'C' behind it was compressed to 15 % of initial area. Similarly, the boom did not leak in the series of runs performed under simultaneously simulated physiological conditions. However, leaks around the boom often occurred when surface tension was

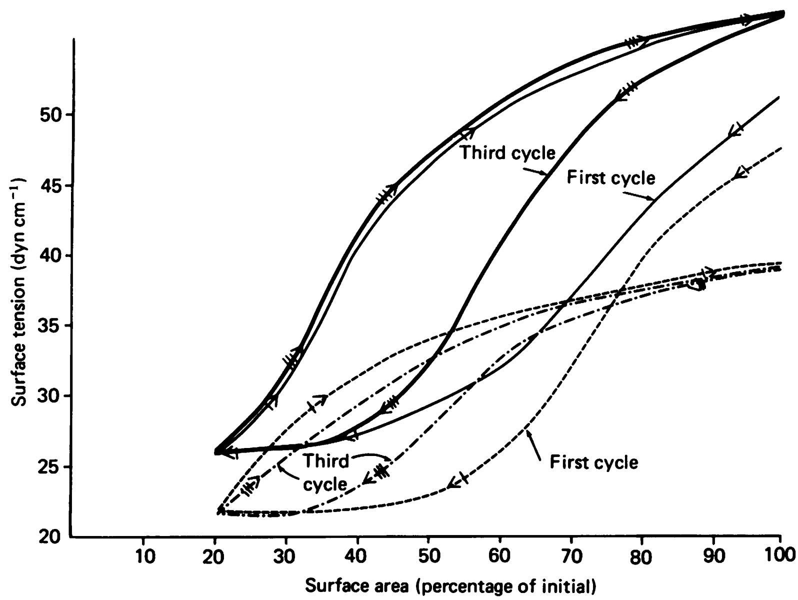

Fig. 3. Typical first and third loops of Langmuir (continuous lines) and Wilhelmy 'B' (dashed lines) monitors simultaneously recording the same film of beef lung extract at 21 ± 1 °C for which the plateau values (for 80 % compression) are given in Table 2. Wilhelmy monitors in pools 'A' and 'C' gave almost constant readings of 71–72 dyn cm^{-1}; unchanged by subsequent compression.

TABLE 2. Surface tensions for beef extract upon film compression to 20 % at 21 °C

Run	Surface tension* (dyn cm^{-1}) Wilhelmy (W)	Langmuir (L)	Contact angle (θ) $\cos^{-1}(W/L)$
No. 1	21	25	33 deg
No. 2	21	26	36 deg
No. 3	24	28	31 deg
No. 4	22	26	32 deg
No. 5	22	26	32 deg
No. 6	22	26	32 deg
No. 7	24	25	16 deg
No. 8	21	26	36 deg
Mean ± S.E. of mean	22·1 ± 0·04	26·0 ± 0·3	31·0 ± 2·2 deg

* Values for the first cycle.

reduced to the low values recorded under laboratory conditions but were easily detected the moment this happened; see Fig. 1.

In all twenty runs with DPL at 21 ± 1 °C, there were leaks or the films collapsed before compression reduced the area to 20 % its original value. The minimum readings on the Wilhelmy balance (pool 'B') for leak-free runs are given in Table 1 together with the 'Langmuir' readings simultaneously recorded on the same film by the Lauda balance. When testing a variety of conditions, the lowest Langmuir reading obtained was 6·9 dyn cm^{-1} for 75 % compression of a DPL film at 35 °C, at which the Wilhelmy reading was 1·1 dyn cm^{-1}, corresponding to a contact angle of 81 deg calculated according to eqn. (2). The loop for that run is shown in Fig. 2.

TABLE 3. Surface tension at 75 % of initial area for first and third cycles of DPL and dog lavage extract under physiological conditions

		First cycle			Third cycle		
		Surface tension (dyn cm^{-1})			Surface tension (dyn cm^{-1})		
Run	Surfactant film	Wilhelmy (W)	Langmuir (L)	Contact angle $\cos^{-1}(W/L)$	Wilhelmy (W)	Langmuir (L)	Contact angle $\cos^{-1}(W/L)$
No. 1	DPL	15	20	41 deg	17	21	36 deg
No. 2	DPL	22	25	28 deg	22	26	32 deg
No. 3	DPL	15	24	51 deg	16	31	59 deg
No. 4	DPL	19	24	38 deg	19	25	41 deg
No. 5	DPL	18	22	35 deg	17	22	39 deg
No. 6	DPL	19	25	41 deg	19	27	45 deg
No. 7	DPL	16	22	43 deg	17	24	45 deg
No. 8	DPL	20	25	37 deg	20	27	42 deg
Mean ± S.E. of mean		18·0 ± 0·9	23·4 ± 0·6	39·3 ± 2·4 deg	18·4 ± 0·7	25·4 ± 1·1	42·4 ± 2·8 deg
No. 1	DLE	26	35	42 deg	26	34	40 deg
No. 2	DLE	24	34	45 deg	23	33	46 deg
No. 3	DLE	26	35	42 deg	25	33	41 deg
No. 4	DLE	26	37	45 deg	27	37	43 deg
No. 5	DLE	23	32	44 deg	26	34	40 deg
No. 6	DLE	28	38	43 deg	26	33	38 deg
Mean ± S.E. of mean		25·5 ± 0·7	35·2 ± 0·9	43·5 ± 0·6 deg	25·5 ± 0·6	34·0 ± 0·6	41·3 ± 1·1 deg

DLE: dog lavage extract.

When the extracts from beef lungs were cycled at 21 °C between 100 and 20 % of initial area for the same surface concentration, viz. 0·38 μg cm^{-2}, surface tensions were lower according to both balances and needed more compression to reach a plateau. As in the DPL runs, the Langmuir readings for beef extracts were always higher than the Wilhelmy readings but the difference was less at maximum compression (20 % area). A typical comparison is given in Fig. 3 for both first and third cycles. Plateau values for the first cycle for both instruments (Lauda and Wilhelmy 'B') are given in Table 2. For no compression (100 % of initial area), the contact angle was 47·7 ± 5·2 deg for the eight beef lungs.

Physiological conditions

When DPL films were cycled between 100 and 75 % of initial area for a pH of 7·4 and 100 % humidification at 37 °C, the Langmuir method invariably gave much higher readings than the Wilhelmy method over all degrees of compression for all cycles. The third loop was essentially repeated upon subsequent cycles for eight films and values for 75% compression are given in Table 3 together with contact angles calculated for this point on the basis of eqn. (2). Contact angles for 100% of initial area on the third cycle averaged $54\cdot7 \pm 4\cdot2$ deg. There was a little hysteresis on the third cycle with the surface tension for expansion seldom exceeding that for compression at the same area by more than 5 dyn cm^{-1}. Typical first and third loops

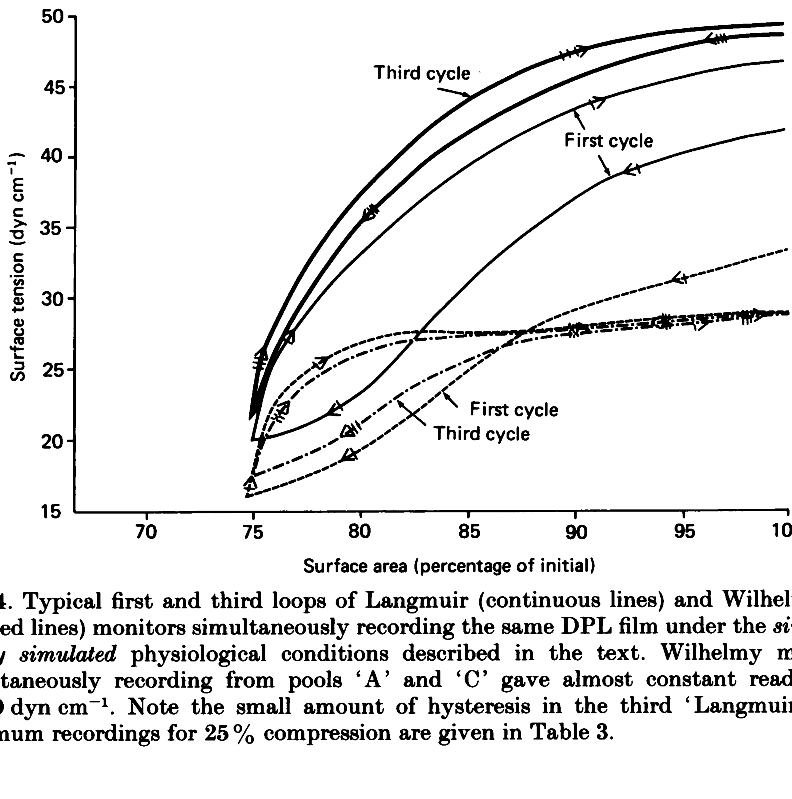

Fig. 4. Typical first and third loops of Langmuir (continuous lines) and Wilhelmy 'B' (dashed lines) monitors simultaneously recording the same DPL film under the *simultaneously simulated* physiological conditions described in the text. Wilhelmy monitors simultaneously recording from pools 'A' and 'C' gave almost constant readings of 69–70 dyn cm^{-1}. Note the small amount of hysteresis in the third 'Langmuir' loop. Minimum recordings for 25% compression are given in Table 3.

recorded by both methods simultaneously are shown in Fig. 4. The extracts from dog lung lavage gave very similar yet rather thinner third loops by the Langmuir method although other loops had a significantly different appearance to those for DPL; see Fig. 5. Langmuir and Wilhelmy values recorded for 25% compression on the first and third cycles are given in Table 3. Maximum Langmuir surface tensions, i.e. those for 100% of initial area, gave a mean value of $43\cdot3 \pm 2\cdot7$ dyn cm^{-1} corresponding to a contact angle on the Wilhelmy flag of $37\cdot1 \pm 3\cdot4$ deg.

DISCUSSION

The results leave little doubt that, for all surfactant films tested, the original Langmuir method gives values of surface tension which are appreciably higher than those determined by the Wilhelmy method when contact angle is ignored, i.e. when

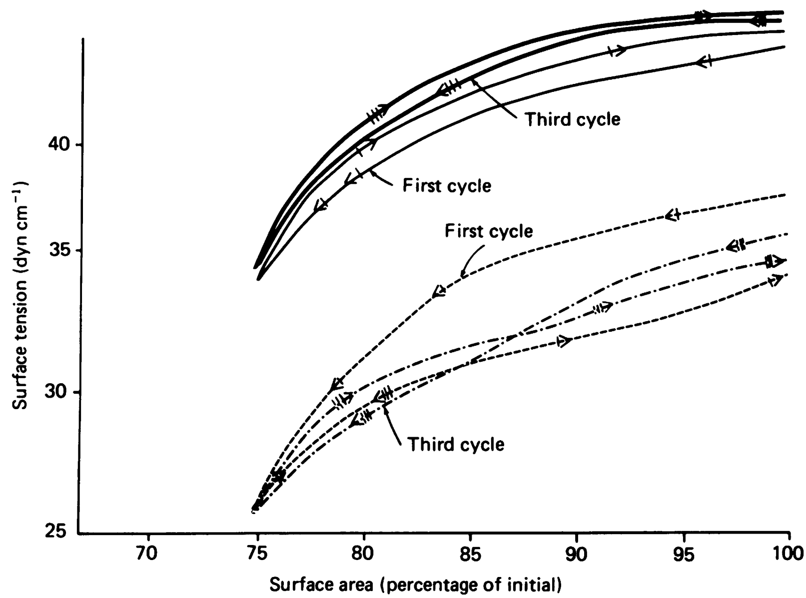

Fig. 5. Typical first and third loops of Langmuir (continuous lines) and Wilhelmy 'B' (dashed lines) monitors simultaneously recording the same film of dog lavage extract under the *simultaneously simulated* physiological conditions described in the text. Wilhelmy monitors simultaneously recording from pools 'A' and 'C' gave almost constant readings of 69–70 dyn cm^{-1}. Note the large difference between Langmuir and Wilhelmy 'B' readings whose lowest values (for 25% compression) are given in Table 3. Note also the small amount of hysteresis in the third Langmuir loop representing true surface tension.

calculated from the Wilhelmy equation (eqn. (2)) assuming θ to be zero. These differences cannot be attributed to leaks since both techniques were monitoring the same film at the same time. Any retention of chloroform by the monolayer should affect both methods equally. In any case, the above observation is based upon data which are most unlikely to involve leaks since, when they occurred Fig. 1, they were immediately detectable. Moreover, subsequent compression of pools 'A' and 'C' to 15% of their respective areas would certainly have caused detectable changes in the surface tension if any surfactant had reached those pools. Even if surfactant had escaped up the Teflon walls undetected despite close observation, the loss should have affected both balances equally. The higher Langmuir reading supports an earlier comparison using the du Noüy method in which Barrow & Hills (1979*a*) have attributed consistently lower Wilhelmy readings to a contact-angle artifact. The values of θ estimated in this study span the range determined on the same platinum plates used in that study (45–70 deg). They are also in agreement with the range of values (42–70 deg) determined for monolayers of DPL deposited on glass and determined directly with a goniometer (Hills, 1984); the standard instrument for measuring contact angles.

The fact remains that the 'Langmuir' values of surface tension determined in this study are appreciably higher than the near-zero values usually quoted for the lung based upon other methods of measurement which need critical review. At least, there is essential agreement with previous results using the ring method (Barrow & Hills, 1979*a*) which is independent of contact angle because it essentially measures the force needed to 'pluck' a ring out of the surface, this force reaching the recorded maximum when the liquid surface is vertical. This force is independent of contact angle but the surface inside of the ring becomes an isolated pool for which it is difficult to compensate for the increase in surface area as the ring is raised.

The methods by which near-zero values have been recorded include the Wilhelmy method, the *in situ* determinations of surface tension in the lung by Schürch *et al.* (1981) and those of Enhorning (1977) using the pulsating bubble technique. The *in situ* data of Schürch *et al.* (1981) are based upon a calibration of the point of spreading of their fluorocarbon droplet across the alveolar surface based on the Wilhelmy technique and therefore dependent on the validity of that method as discussed later. There is the additional point that spreading on a curved surface is easier than that on a flat surface as used in the calibration.

The Wilhelmy technique is particularly popular and every serious user of this method knows that 'greasy' plates give artifactually low readings of surface tension yet few seem to realise that phospholipids make plates 'greasy'. A saline drop will spread over a clean horizontal platinum plate but plates removed from the Langmuir trough at various stages of the cycles in the above studies and placed on the stage of a goniometer show contact angles ranging from 0 to 70 deg. The problem is that one never knows the true contact angle at any stage of the cycle as the meniscus runs up and down the plate, in jumps at the micro level, depositing varying amounts of surfactant which need scraping or a solvent to remove. Some experimentalists prefer to roughen the plate in order to minimize contact angle but recent comprehensive studies on surface roughness by Mason (1978) have shown that it does indeed reduce minimum contact angle but also increases the maximum. This means an increase in contact-angle hysteresis which is a serious error especially when relating surface tension data to compliance hysteresis. These arguments would all seem consistent with the differences between the Langmuir and Wilhelmy results found in this study. Where the Wilhelmy method has been modified by use of a wire frame instead of a flat plate by Brown *et al.* (1959), the minimum values for lung extracts have been higher (10–15 dyn cm^{-1}) and this may reflect the fact that the system is now approximating more closely to the wire ring of the du Noüy method discussed above.

Another method much quoted in support of Wilhelmy values of near-zero surface tension is one by which surfactant is recruited from the aqueous phase to the surface of a bubble pulsating between known diameters (Enhorning, 1977). Pulsation is produced by a reciprocating piston at a frequency at which the various components and fluid columns had apparently not been checked for resonance which is a frequent complication in hydraulic systems. It is very difficult to see how such low γ values could be recorded with confidence when the kinetic energy of the fluid, and possibly the transducer also, are so large by comparison with the surface energy of the bubble being measured. Considerations such as these may explain why Enhorning (1977) found unacceptably high values for water which he did, indeed, attribute to mechanical factors associated with the transducer. Resonance and inertial factors in general might be eliminated by switching from a hydraulic to a pneumatic system in using a two-sided rather than a one-sided bubble, but this author could not prevent the bubble from bursting. However, two-sided bubbles expressed from lung tissue as a foam have been found to be exceptionally stable with Pattle (1958) pointing out that this indicated extremely low surface tension. This argument would be perfectly valid if the fluid from lung tissue did not contain protein which can stabilize bubbles by a mechanism involving a macromolecular skeleton (Cumper, 1953). This is exploited commercially in foam stabilizers, as used in some British beers, and can be effective even with liquids of quite high surface tension.

Over-all, it is probably fair to say that, although the surface tension values determined by the Langmuir method conflict with those of several other methods, each of those is open to major criticism. This applies especially to the Wilhelmy method where our readings (Table 1) would have agreed with those studies if we had chosen to ignore the contact angle.

If the Langmuir readings reflect true surface tension, then the implications need to be considered in two steps. The first is that the lowest recorded value of 6·9 dyn cm^{-1} is still very low and compatible with the bubble model of the alveolus for large mammals such as man for which the Laplace equation gives $\Delta P = 9$ cmH_2O, assuming $r = 150$ μm in eqn. (1). However, it becomes difficult to envisage ventilation and homoeostasis in animals such as the bat and shrew ($\Delta P = 73$ cmH_2O) or even the rabbit ($\Delta P = 24$ cmH_2O).

Even values as low as 7 dyn cm^{-1} (Table 1) are only obtainable under conditions which are very difficult to justify as occurring in the lung (Barrow & Hills, 1979*b*). If film compression is reduced to the 25 % level (from 100 to 75 % area) which Pattle (1977) has considered close to the upper limit, then surface tension is only reduced to 34·0 dyn cm^{-1} for dog lung extract or to 25·4 dyn cm^{-1} for DPL (see Table 3) which are close to values determined by the du Noüy method of 24·3 dyn cm^{-1} for DPL on Ringer solution and 33·7 dyn cm^{-1} for DPL on dog serum (Barrow & Hills, 1979*b*). Substituting the lowest of these values (24·3 dyn cm^{-1}) in eqn. (1), we now obtain $\Delta P = 32$ cmH_2O for man ($r = 150$ μm) which is totally incompatible with normal physiological function. The same conclusion is reached using Wilhelmy values recorded under these conditions. The above values for ΔP are conservative since, as Tierney (1974) points out, one should really base the calculation on those areas at the septal corners where the liquid surface has the greatest curvature (lowest r values), giving the largest pressure differentials. When this is done, the ΔP values obtained are even less compatible physiologically and the many arguments put forward by Tierney (1974) for a low alveolar surface tension based on a continuous aqueous hypophase can be turned around as criticism of the model on which those deductions were made.

The foregoing discussion leads to the conclusion that it would seem unlikely that the alveolus could be lined with a continuous aqueous hypophase on which surfactant imparts γ values in the region recorded in this study by the Langmuir method. This leads to the concept that the net contractile force may be determined by other factors, but still related to the surface because it is greatly reduced upon liquid filling the lung. One possibility is that the aqueous hypophase is really discontinuous because surfactant directly adsorbed to the alveolar wall provides a water repellent coating disrupting any continuous (bubble) lining which might otherwise form (Hills, 1982*a*, 1984). Another possibility is that the surfactant on the hypophase forms a condensed monolayer of DPL with elimination of any other components to give a rigid 'shell' capable of withstanding alveolar collapse (Bangham *et al.* 1979). The surfactant is now in the gel phase and has been demonstrated on the Langmuir trough to resist compression with a force equal to the surface tension of water, i.e. reducing the net contractile force to near zero. This would seem feasible if the 'shell' of condensed surfactant were as effective in resisting collapse in a three-dimensional structure as the monolayers displayed in two dimensions in the *in vitro* studies. From the basic theory of thin-walled structures, this would only seem likely if the contours were smooth which they could cause by squeezing out intervening water and moulding tissue where necessary. This may explain the remarkable surface continuity between wet and dry areas of the alveolar surface seen in some electron micrographs (Gil, 1983). It is possible that direct contact of the 'shell' with epithelium and the ideal orientation of its molecules could lead to adsorption which would, in effect, come back to a model very similar to that based upon a discontinuous aqueous hypophase. Whether the dry areas observed between the 'pools' on the alveolar surface in electron micrographs are reached through 'shells' (Bangham *et al.* 1979) or direct adsorption (Hills, 1982 *a*), the resulting elimination or virtual elimination of the liquid layer adding to the blood–air barrier is an appreciable advantage to gas transfer. Other factors have been discussed elsewhere (Hills, 1982*a*, *b*).

In conclusion, the choice of mechanical model to be used for the alveolus depends very much upon the surface contractile forces acting and the great difficulties experienced in using any method to measure them. However, it is felt that the original Langmuir method raises the fewest objections although the results differ from most other methods, especially those used to obtain results much quoted in favour of the popular bubble model.

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REFERENCES

BANGHAM, A. D., MORLEY, C. J. & PHILLIPS, M. C. (1979). The physical properties of an effective lung surfactant. *Biochimica et biophysica acta* **573**, 552–556.

BARROW, R. E. & HILLS, B. A. (1979*a*). A critical assessment of the Wilhelmy method in studying lung surfactants. *Journal of Physiology* **295**, 217–227.

BARROW, R. E. & HILLS, B. A. (1979*b*). Surface tension induced by dipalmitoyl lecithin *in vitro* under physiological conditions. *Journal of Physiology* **297**, 217–227.

BROWN, E. S., JOHNSON, R. P. & CLEMENTS, J. A. (1959). Pulmonary surface tension. *Journal of Applied Physiology* **14**, 717–720.

CLEMENTS, J. A., HUSTEAD, R. F., JOHNSON, R. P. & GRIBETZ, I. (1961). Pulmonary surface tension and alveolar stability. *Journal of Applied Physiology* **16**, 444–450.

CLEMENTS, J. A. & TIERNEY, D. F. (1965). Alveolar instability associated with altered surface tension. In *Handbook of Physiology: Respiration*, vol. II, ed. FENN, W. O. & RAHN, H., pp. 1565–1583. Washington: American Physiological Society.

CUMPER, C. W. N. (1953). The stabilization of foams by proteins. *Transactions of the Faraday Society* **49**, 1360–1369.

DICKIE, K. J., MASSARO, G. D., MARSHALL, V. & MASSARO, D. (1972). Amino acid incorporation into protein of a surface-active lung fraction. *Journal of Applied Physiology* **34**, 606–614.

ENHORNING, G. (1977). Pulsating bubble technique for evaluating pulmonary surfactant. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology* **43**, 198–203.

FOLCH, J., LEES, M. & SLOANE-STANLEY, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

FROSONOLO, M. F., CHARMS, B. L., PAWLOWSKI, R. & SLIVKA, S. (1970). Isolation, characterization, and surface chemistry of a surface-active fraction from dog lung. *Journal of Lipid Research* **11**, 439–457.

GAINES JR, G. L. (1966). Transfer of monolayers to solids. In *Multilayers. Insoluble Monolayers at Liquid–Gas Interfaces*, chap. 8, pp. 326–346. New York: Wiley.

GIL, J. (1983). Alveolar surface, intra-alveolar fluid pools and respiratory volume changes. *Journal of Applied Physiology: Respiration, Environmental and Exercise Physiology* **54**, 321–323.

GLUCK, L., MOTOYAMA, E. K., SMITS, H. L. & KULOVICH, M. V. (1967). The biochemical development of surface activity in mammalian lung. *Pediatric Research* **1**, 237–246.

HARKINS, W. D. & ANDERSON, T. F. (1937). I: A simple, accurate film balance of the vertical type for biological and chemical work and a theoretical and experimental comparison with the horizontal type. II: Tight packing of a monolayer by ions. *Journal of the American Chemical Society* **59**, 2189–2197.

HILLS, B. A. (1982*a*). Water repellency induced by pulmonary surfactants. *Journal of Physiology* **325**, 175–186.

HILLS, B. A. (1982*b*). What forces keep lung air spaces dry? *Thorax* **37**, 713–717.

HILLS, B. A. (1984). 'De-watering' capabilities of surfactants in human amniotic fluid. *Journal of Physiology* **348**, 369–381.

HILLS, B. A. & NG, Y. L. (1974). Significance of the contact angle in studies of lung surfactant. *Journal of Physiology* **241**, 52–53*P*.

KING, R. J. & CLEMENTS, J. A. (1972*a*). Surface active materials from dog lung. I. Method of isolation. *American Journal of Physiology* **223**, 707–714.

KING, R. J. & CLEMENTS, J. A. (1972*b*). Surface active materials from dog lung. II. Composition and physiological correlations. *American Journal of Physiology* **223**, 715–726.

KLAUS, N. H., CLEMENTS, J. A. & HAVEL, R. J. (1961). Compositions of surface-active material isolated from beef lung. *Proceedings of the National Academy of Sciences of the U.S.A.* **47**, 1858–1859.

LANGMUIR, I. (1917). The constitution and fundamental properties of solids and liquids. II. Liquids. *Journal of the American Chemical Society* **39**, 1848–1905.

MASON, S. G. (1978). Wetting and spreading – some effects of surface roughness. In *Wetting, Spreading and Adhesion*, ed. PADDAY, J. F., p. 323. London: Academic.

MENDENHALL, R. M. & STOKINGER, H. E. (1962). Films from lung washings as a mechanism model for lung injury by ozone. *Journal of Applied Physiology* **17**, 28–32.

PATTLE, R. E. (1958). Properties, function and origin of the alveolar lining. *Proceedings of the Royal Society* B **148**, 217–240.

PATTLE, R. E. (1977). The relation between surface tension and area in the alveolar lining film. *Journal of Physiology* **269**, 591–604.

RADFORD, E. P. (1957). Recent studies of mechanical properties of mammalian lungs. In *Tissue Elasticity*, ed. REMINGTON, J. W., pp. 177–190. Washington: American Physiological Society.

ROUSER, G., SIAKOTOS, A. N. & FLEISCHER, S. (1966). Quantitative analysis of phospholipids by thin-layer chromatography and phosphorus analysis of spots. *Lipids* **1**, 85–86.

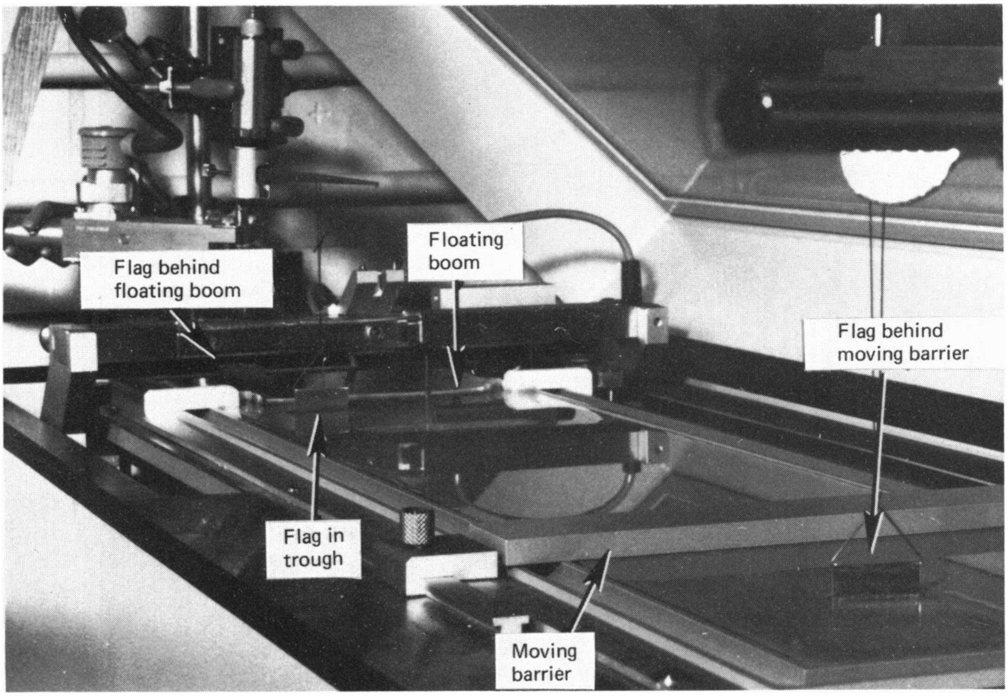
SCARPELLI, E. M. (1968). *The Surfactant System of the Lung*, p. 140. Philadelphia: Lea & Febiger.

SCARPELLI, E. M., CHANG, S. J. & COLACICCO, G. (1970). A search for the surface-active lipoprotein. *American Review of Respiratory Diseases* **102**, 285–289.

SCHÜRCH, S., GOERKE, J. & CLEMENTS, J. A. (1976). Direct determination of surface tension in the lung. *Proceedings of the National Academy of Sciences of the U.S.A.* **73**, 4698–4702.

STEIM, J. R., REDDING, R. A., HAUCK, C. T. & STEIM, M. (1969). Isolation and characterization of lung surfactant. *Biochemical and Biophysical Research Communications* **34**, 434–440.

TIERNEY, D. F. (1974). Lung metabolism and biochemistry. *Annual Review of Physiology* **36**, 209–231.


Flag behind
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barrier

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VON NEERGAARD, K. (1929). Neue Auffasungen uber einen Grundbegriff der Atemmechanik die Retraktionskraft der Lunge, abhangig von der Oberflachenspannung in den Alveolen. *Zeitschrift für die Gesamtel Experimentelle Medizin* **66**, 373–394.

WILHELMY, L. (1863). Uber die Abhanigkeit der Capillaritats-Constanten des Alkols von Substanz und Gestalt des benetzten festen Korpers. *Annalen der Physik Leipzig* **119**, 177–217.

EXPLANATION OF PLATE

The Langmuir trough shown with the surface divided into three pools by the floating boom and the moving barrier, a Wilhelmy flag dipping into each pool. A horizontal force transducer measures the net difference in the surface forces across the floating boom according to the original method of Langmuir (1917).